Chapter 1
Introduction

1. Introduction

The number of poorly soluble drugs is steadily increasing. About 40% of the drugs in the development pipe lines and approximately 60% of the drugs coming directly from chemical synthesis are poorly soluble. However, there are also drugs which show low permeability that can be caused by the efflux pumps present in the gastrointestinal drugs, e.g. p-glycoprotein. Consequently most of them exhibit a poor oral bioavailability because in general low solubility and low permeability is correlated with poor oral bioavailability. The currently available conventional dosage forms are mostly immediate release, require frequent dosing and are associated with non – compliance. The challenge is to create novel drug delivery systems which can overcome these drawbacks associated with conventional therapy. The proposed novel delivery system should not only dissolve drug fast but should be combined with a technology to improve bioavailability.

Many promising antiviral agents unfortunately have disadvantageous physicochemical properties that lead to poor bioavailability and biodistribution (Briesen et al., 2000). One basic requirement for the successful use of any drug against retrovirus (e.g. HIV) -related diseases is to have sufficient bioavailability (Robins and Plattner, 1993). To achieve successful therapy against viral disease for orally administered drugs, it is essential to be adequately and consistently absorbed. High dose of drugs has to be taken by patient since treatment objective is to completely inhibit viral proliferation, an effect which is proportional to drug concentration. The achievement of high plasma concentration of antiretroviral drugs is considered beneficial when a) inhibition of viral suppression in all the body compartments is required, b) the development of resistance is observed and c) maintenance of higher 'trough' concentrations is desired, which may better prevent the presence of replication .

The 2006 estimates suggest national adult HIV prevalence in India is approximately 0.36 %, amounting to between 2 and 3.1 million people (http://nacoonline.org, 2006). More men are HIV positive than women. Nationally, the prevalence rate for adult females is 0.29%, while for males it is 0.43 %. This means that for every 100 people living with HIV and AIDS, 61 are men and 39 women. Prevalence is also high in the 15-49 age group (88.7 percent of all infections), indicating that AIDS still threatens the cream of society, those in the prime of their working life. In absolute numbers, India's AIDS figure is still substantial. It is the third largest in the world, and remains the largest in Asia.

The first Protease inhibitor approved for treatment of patients with Acquired Immunodeficiency Deficiency Syndrome was Saquinavir (SQ). It displays extreme potency in vitro against HIV-1 and HIV-2 in various cell culture systems, with 50% inhibitory concentrations (IC 50s) in the nanomolar range (Craig et al., 1991; Roberts et al., 1990). It is effective in reducing viral load and is well tolerated. Saquinavir mesylate has a low oral bioavailability (approximately 4– 8%) caused by: (1) an important hepatic first pass metabolism, (2) a limited absorption due to a poor water solubility (Noble and Faulds, 1996), 3) Low permeability due to membrane bound efflux of P glycoprotein. Following single 600 mg doses, the relative bioavailability of saquinavir compared to saquinavir administered as saquinavir mesylate was estimated as 331% (95% CI 207-530%) (www.rocheusa.com, 2007). Several reports mentioned that low and variable oral bioavailability of SQ is primarily caused by membrane bound efflux proteins like P-glycoprotein and multi-drug resistance proteins (MRPs) and partly due to CYP3A4 mediated metabolism (Dupre et al., 1995).

Adefovir is a nucleoside reverse transcriptase inhibitor approved by the FDA in November 1998 for the treatment of HIV infections. Adefovir has activity against retro-, herpes- and hepadnaviruses but, like other Adenine Nucleoside Phosphate (ANP) analogues, displays poor oral bioavailability as a result of the highly anionic phosphonate moiety which limits transport into intestinal cells (Shaw and Cundy, 1993). Ester derivatives of Adefovir (Adefovir Dipivoxil) and other ANP analogues (with a lipophilic molecule added to the phosphonate group) have been synthesized to improve cellular uptake (Stuart Noble et al, 1999). Adefovir dipivoxil diffuses passively into cells, unlike adefovir, which appears to require an endocytosis- like transport process (Palu et al., 1991).The intestinal permeability of adefovir is enhanced when the drug is provided as adefovir dipivoxil rather than free adefovir though oral bioavailability of adefovir from 10 mg adefovir dipivoxil is 59 % in human (www.emea.europa.eu, 2006). The *in vitro* permeability of adefovir dipivoxil across Caco-2 intestinal mucosal monolayers was 7.9 x 10^{-6} compared with 1.0 x 10^{-6} cm/sec for unmodified adefovir (Cihlár et al., 1995). The oral bioavailability of adefovir ranged between 34 - 47 % in rats (Shaw et al., 1997) and between 21 to 35 % in monkeys (Cundy et al., 1994).

The development of novel oral delivery systems allowing increased dissolution rates for highly lipophilic drugs is generating growing interest. Different strategies described to achieve this objective include the use of surfactants, cyclodextrin, nanoparticles, solid

dispersions, lipids and permeation enhancers. Particulate delivery systems have also been extensively studied. Among the particulate systems, Solid Lipid Nanoparticles (SLNs) and Nanosuspensions have received tremendous academic and commercial interest. SLNs have shown great promise in terms of both improved dissolution as well as controlled release while drugs which show poor solubility in aqueous media can be easily formulated into nanosuspensions to improve dissolution and bioavailability.

SLN are systems consisting of drug (s) incorporated in solid lipid matrices of nanometer size. Like other formulations, such as microemulsions or submicron emulsions, reduction in particle size is a key factor for improving the oral performance of drugs when incorporated in SLNs (Lian Dong Hu et al., 2004). Decrease in particle size results in increase in surface area and saturation solubility which in turn increases release rate of the drug and allows it to reach high concentration in the Gastrointestinal Tract (GIT). Due to their small particle size, SLNs may exhibit bioadhesion to the GIT wall or enter the intervillar spaces, thus increasing their residence time in the GIT. This increase in adhesion will result in enhanced bioavailability. Reduction in particle size leads to increase in area under plasma versus time curve, enhanced onset of action, peak drug level, reduced variability, reduced fed/fasted effects. Recently, increasing attention has been focused on SLNs because of advantages like controlled release, no problems with respect to large scale production, increased drug stability, feasibility in incorporation of lipophilic & hydrophilic drugs, avoidance of organic solvents and since physiological lipids are chosen, low or no toxicity is expected. The use of solid lipids as a matrix for drug delivery is well known in the form of lipid pellets in oral drug delivery (Mucosolvan™, retard capsules) and in the form of microparticles produced by various techniques (Speiser, 1990; Eldem et al., 1991). Solid lipid nanoparticles possess solid matrices for the controlled release of drugs, avoiding the burst release as obtained with fat emulsions. The pharmacokinetics of a drug can be changed greatly when the drug is incorporated into nanoparticles, owing to the controlled release of the drug from the nanoparticles and the alteration in body distribution of the drug through incorporation into nanoparticles. A prolonged release was first obtained when studying prednisolone loaded SLN (Muhler and Mehnert, 1998). Antiviral therapy requires high, prolonged plasma & tissue drug levels where viral replication could be controlled. SLN as prolonged release dosage form may simplify therapy, increase patient adherence and potentially provide

more durable control of viral replication in plasma, therefore maintaining the long term efficacy of the regimen (Taburet et al., 2003).

Nanosuspensions are sub-micron colloidal dispersions of pure particles of drug, which are stabilized by surfactants. They are distinguished from nanoparticles, which are polymeric colloidal carriers of drugs, and from solid lipid nanoparticles, which are lipidic carriers of drugs. Nanosuspensions have revealed their potential to tackle the problems associated with the delivery of poorly water soluble and poorly water and lipid soluble drugs, and are unique because of their simplicity and the advantages they confer over other strategies. Attractive features, such as increased dissolution velocity, increased saturation solubility, versatility in surface modification and ease of post-production processing, have widened the applications of nanosuspensions for various routes. The applications of nanosuspensions in parenteral and oral routes have been well investigated and applications in pulmonary and ocular delivery have been realized.

Like other formulations, such as microemulsions or submicron emulsions, reduction in the particles size is a key factor for improving the peroral performance of poorly soluble drugs. The surfactants may improve affinity between lipid particles and intestinal membrane or increase permeability of the intestinal membrane. By reducing size of particles to the sub-micron level, the uptake of intact particles has been shown to occur preclinically (Florence and Hussain, 2001; Lark et al., 2001) by mechanisms involving M-cells in Peyer's patches of the gastrointestinal lymphoid tissue (Jani et al., 1990). This uptake pathway communicates with the mesenteric lymph ducts, and empties via thoracic duct into the systemic blood circulation. The low drug uptake by this pathway might be enhanced by transporter receptors on the intestinal epithelium.

These approaches will provide a route for Saquinavir SLNs for targeting sanctuaries of lymphatic-mediated diseases. SLNs may provide lipid protection to Saquinavir and Adefovir Dipivoxil from chemical and enzymatic degradation, thereby delaying the in vivo metabolism. SLNs formulations exhibit high bioadhesion and remain for a long time in the gastrointestinal tract. This increase in adhesion will result in enhanced bioavailability of Adefovir Dipivoxil SLNs.

In case of Saquinavir and Adefovir Dipivoxil Nanosuspension, their small size and increased surface area may lead to an increased dissolution rate and increased bioavailability. In addition, increased surface area and decreased particle size may lead to increased mucoadhesion, which can increase gastrointestinal transit time and lead to

increased bioavailability. This enhancement in bioavailability will lead to a subsequent reduction in dose of Saquinavir and Adefovir, rendering the therapy cost-effective and obliterating any undue drug dumping in the body.

Aims & Objectives:

Aim of the present project was to develop Solid lipid Nanoparticles and Nanosuspensions of Saquinavir and Adefovir Dipivoxil for improvement of oral bioavailability with following objectives:-

- To formulate dosage forms with enhanced dissolution which ultimately increase absorption and hence bioavailability.
- To formulate sustained release formulations which will maintain prolonged drug levels, control therapy and also drug dose.
- To improve patient compliance by reducing dosing frequency.
- To provide cost effective therapy for treatment of viral infection

In the present investigation, we propose Nanoparticulate delivery systems for Saquinavir and Adefovir Dipivoxil for improvement of oral bioavailability.
It is envisaged that the proposed delivery systems will improve solubility and permeability of drug which will result in increased bioavailability. It is expected to provide sustained release of drugs which will reduce the frequency of administration of conventional dosage form and will improve patient compliance.

Plan of work:

1. Preformulation study – Screening of excipients and characterization of API
2. Formulation of Nanoparticulate systems for Saquinavir and Adefovir Dipivoxil: Solid lipid Nanoparticle and nanosuspension.
3. Optimization of process and formulation variables.
4. In vitro characterization, evaluation and drug release
5. Stability studies – Short term and Long term
6. In vivo study of Optimized formulation.

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